Remarks

An Application Data Sheet (ADS) is submitted herewith setting forth the mailing or post office address of each inventor including the Zip Code designation.

Claims 33 and 34-41 were rejected under 35 USC §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 33 have been amended by replacing the term "desaturase-related" with "hydroxylase." Also the term "hydroxylase" was added to part (b) of claim 33. Support for this can be found in the specification on page 19 at lines 8-18. Thus, no new matter has been added.

Claim 33 was also amended to delete the second occurrence of "of a part thereof." No new matter has been added.

Claim 42 was amended to correct an obvious typographical error.

The specification has been amended to correct an obvious typographical error.

Withdrawal of the rejection of claims 33 and 34-41 under 35 USC §112, second paragraph, is respectfully requested in view of the above amendments and discussion.

Submitted herewith is a terminal disclaimer to address the rejection of claims 1 and 33-44 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-13 of U.S. Patent No. 6,372,965.

Claims 1 and 33-44 were rejected under 35 USC §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, has possession of the claimed invention.

The concern raised on page 5 of the Office Action is that the "specification does not disclose what structural features would be conserved in the claimed sequences that would result in the claimed enzyme activity that is desaturase, desaturase-related or that catalyzes a reaction at carbon positions 6 and 7 numbered from the methyl end of an 18 carbon long fatty acyl chain. Nor does the specification describe the structural features required for a nucleic acid fragment to confer antisense or co-suppression of an endogenous desaturase gene. . . Therefore, given the lack of written description in the specification with regard to the structural and physical characteristics of the claimed composition, one skilled in the art would not have been in possession of the genus claimed at the time this application was filed."

Attention is kindly invited to Claim 7 of the '965 patent that recites certain motifs (i.e., amino acid sequences) which the amino acid sequence comprising the enzyme encoded by the corresponding nucleotide sequence can contain. Support for these motifs can be found in Table 7 on pages 47 and 48 of the specification. These motifs provide sufficient information to direct the synthesis of primers for use in PCR or for the hybridization protocols

to recover DNA fragments corresponding to these proteins. For example, the corn cDNA encoding seed microsomal delta-12 fatty acid desaturase was isolated using PCR to obtain a fragment that was later useful as a probe for the full-length clone. This is discussed in the specification on page 38, line 4 through line 13 on page 39 and on page 75, line 25 through line 18 on page 77.

Applicants respectfully submit that in view of the above discussion, it is clear that Applicants were in possession of the claimed genus at the time the application was filed.

Claims 1 and 33-44 were rejected under 35 USC §112, first paragraph, on the ground that "it would require undue experimentation to identify which other sequences encompassed by the claims have desaturase activity, desaturase-related activity, antisense or co-suppression activity, and furthermore, what type of desaturase activity that would be. . . ."

Previously submitted was a copy of the Declaration of Dr. Anthony Kinney which shows unequivocally the close evolutionary/structural relationship of delta-12 desaturase and "desaturase-related" proteins such as a hydroxylase. In addition, Dr. Kinney's declaration discusses the article of Broun et al., in "Physiology, Biochemistry and Molecular Biology of Plant Lipids," Williams, J. P. et al., eds., Kluwer Academic Publishers, Dordrecht, 1997, pages 342 - 344 (copy attached to the copy of Dr. Kinney's Declaration) which further supports the relationship between a delta-12 desaturase and desaturase-related enzymes such as a hydroxylase. Figure 1 in Broun et al. illustrates this point. The wild-type form *of L. fendleri* "hydroxylase" is shown to produce both polyunsaturated and hydroxylated structures (presumably from oleate). Thus, it is not possible to say that delta-12 desaturases and 12-hydroxylases constitute distinct classes of proteins, because this single protein possesses a combination of the properties, structural features, or functions that would be used to distinguish the classes. The authors of the article used site-directed mutagenesis to introduce only six amino acid substitutions into the wild-type protein. In yeast strains expressing the mutated hydroxylase gene, ratios of 18:2 to ricinoleic acid levels were more than 30-fold higher than in control strains expressing the wild-type gene. Also provided herewith is a copy of an article by Broun et al., entitled "Catalytic Plasticity of Fatty Acid Modification Enzymes Underlying Chemical Diversity of Plant Lipids" (*Science* **282**:1315-1317 (1998)). The authors of this article further characterize the subtle changes in protein sequence/structure that are responsible for the skewing of catalytic function to either predominantly hydroxylase or predominantly desaturase, as judged by the relative proportions of hydroxylated or unsaturated product. Among other findings, these authors report that as few as four amino acid substitutions were necessary to convert the *Arabidopsis* FAD2 gene product from a strict desaturase to a mixed desaturase/hydroxylase (see paragraph bridging pages 1316 and 1317).

The specification clearly teaches the isolation and characterization of a variety of plant delta-12 desaturases and a 12-hydroxylase as well as a means for distinguishing these proteins from plant desaturases of differing specificity, such as, delta-15 desaturases.

Five different delta-12 fatty acid desaturase genes and a gene for a plant 12-hydroxylase were isolated from divergent plant species representing both monocots and dicots. Applicants used a single desaturase gene (from *Arabidopsis*) as a probe to isolate a number of delta-12 desaturase genes from other plants.

Applicants have shown that these genes can be used to alter the fatty acid profile of transformed host plants and plants cells by:

1) restoring desaturase activity in a plant that is deficient in this activity as is shown on page 32, line 22 through page 35, line 2; and

2) reducing desaturase activity in transformed plants by genetic suppression of gene expression as in illustrated in Example 6.

It is respectfully submitted that one of ordinary skill in the art could easily determine, by sequence comparison, whether a cloned sequence encoded a delta-12 desaturase or 12-hydroxylase enzyme and could reasonably expect to successfully alter the fatty acid profile of a plant transformed with a gene falling within the scope of the claimed invention.

Parenthetically, it should be noted that the original Declaration can be found in the file of Applicants' Assignee's Application No. 08/256,047 filed on October 7, 1994 the subject matter of which is related to the present case.

In view of the above discussion, Applicants respectfully request withdrawal of the rejection of the claims under 35 USC §112, first paragraph.

Claims 33-35 and 37-41 were rejected under 35 USC 102(b) as being anticipated by Wada et al. (Nature 347, 13 September 1990, pp. 200-203).

Wada et al. describe enhancement of chilling tolerance of a **cyanobacterium** by genetic manipulation of fatty acid desaturation. A chilling sensitive cyanobacterium was made more tolerant to chilling by introducing a gene for a plant-type desaturation obtained from a chilling –resistant cyanobacterium. The subject gene of Wada et al. is the delta-12 desaturase from *Synechocystis* (GenBank Accession D13778), also known as the *desA* function or gene.

The instant specification compares the coding region of the *desA* gene and its translation product with the corresponding plant sequences that form the basis of claims 33-35 and 37-41. The Examiner's attention is kindly drawn to pages 44 through 47 of the specification, where the comparisons of these sequences by the method of Needleman et al. are presented in tabular form and are commented upon in the accompanying text. Also provided herewith are visual alignments of the *desA* DNA and protein sequences with the corresponding full-length sequences for desaturases and hydroxylase from the specification. These accessory comparisons were performed using the method of J. Hein (for DNA sequences) and CLUSTALW (for protein sequences). Also provided herewith in tabular form are the identity values that result from these alignments. It is readily seen that the encoded product of the *desA* gene is less than 30% identical to any of the gene products that form the

basis for the claims in question. It is also clear that irrespective of the method employed in these comparisons, the nucleic acid sequence encoding the desA desaturase is less than 50% identical to the coding sequences of the plant desaturases or hydroxylase that are referenced in the claims.

In contrast, the instant invention concerns an isolated nucleic acid fragment comprising a nucleic acid sequence selected from the group consisting of:

(a) a nucleic acid sequence encoding a fatty acid desaturase or a hydroxylase **plant** enzyme with an amino acid identity of 50% or greater to the polypeptide encoded by any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15, or

(b) a nucleic acid sequence or a part thereof which is useful in antisense inhibition or sense suppression of endogenous desaturase or hydroxylase activity in a transformed **plant** wherein the nucleic acid has an identity of 80% or greater to any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15. (Emphasis added.)

In view of the above discussion, Applicants respectfully request withdrawal of the rejection of the claims under 35 USC §102(b). It is respectfully submitted that the case is now in form for allowance which allowance is respectfully requested.

A petition for a three (3) month extension of time accompanies this response.

Please charge any fees associated with the filing of this response and the petition for the extension of time to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company). If the fee is insufficient or incorrect, please charge or credit the balance to the above-identified account.

Respectfully submitted,

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Enclosures